Rheumatology 2009;48:233–242
Advance Access publication 16 January 2009
doi:10.1093/rheumatology/ken466

Statins inhibit C-reactive protein-induced chemokine secretion, ICAM-1 upregulation and chemotaxis in adherent human monocytes

F. Montecucco[1], F. Burger[1], G. Pelli[1], N. K. Poku[1], C. Berlier[1], S. Steffens[1] and F. Mach[1]

Objectives. We have recently shown that CRP induces chemokine secretion and adhesion molecule up-regulation in human primary monocytes cultured in adherence. Given the increasing evidence on direct immunomodulatory properties of statins, we investigated their possible anti-inflammatory role on CRP-treated human monocytes.
Methods. Monocytes were isolated by Ficoll–Percoll gradients and cultured in adherence to polystyrene. Chemokine secretion and adhesion molecule expression were detected by ELISA and flow cytometry. Migration assays were performed in modified Boyden chambers. Intracellular kinase activation was assessed by western blot.
Results. Treatment with simvastatin or atorvastatin decreased CRP-induced release of CCL2, CCL3 and CCL4. In addition, both statins reduced CRP-induced intercellular adhesion molecule (ICAM-1) up-regulation, but had no effects on CD11b and CD18. Treatments with 1 μM simvastatin or atorvastatin significantly inhibited monocyte migration in response to CRP. CD32 and CD64 (CRP receptors) expression on monocytes was not affected by statins. Statin-induced inhibition of CRP-mediated chemokine secretion, ICAM-1 up-regulation and migration occurred through the inhibition of extracellular signal-regulated kinase (ERK) 1/2. Treatment with L-mevalonate or farnesylpyrophosphate, but not geranylgeranyl-pyrophosphate reversed the statin-induced effect on CRP-mediated functions and ERK 1/2 phosphorylation, confirming that statins blocked CRP-induced ERK 1/2 phosphorylation through the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.
Conclusions. Statins inhibited CRP-induced chemokine secretion, ICAM-1 up-regulation and migration in human adherent monocytes, through the inhibition of HMG-CoA reductase-ERK 1/2 pathway. This pathway could represent a very promising target to reduce CRP-induced activities in monocyte-mediated diseases, such as atherosclerosis or RA.

Key words: Inflammation, Rheumatoid arthritis, Cytokines and inflammatory mediators, Cell receptor–ligand interaction, Signalling and activation, Monocytes.

Introduction

Emerging evidence shows that chronic inflammatory processes characterizing RA increase and accelerate atherogenesis [1]. The excess of cardiovascular mortality and morbidity in RA patients strongly supports the strong association between these two inflammatory diseases [2–5]. Common inflammatory mediators orchestrate pathophysiological processes in RA and atherosclerosis [6]. Among several inflammatory factors, clinical trials and basic research support a role for CRP as a pro-inflammatory factor, which powerfully predicts future cardiovascular events [7–11]. In particular, the chronic increase of CRP serum levels in RA subjects can directly contribute to atherogenesis, by activating several inflammatory and vascular cell types (including leucocytes, endothelial cells and smooth muscle cells) [12, 13]. In the last decade, CRP secretion has been shown not only by the hepatocytes, but also within atherosclerotic lesions. In support of a 'paracrine' intraplaque activity of CRP, we have recently shown that adherence to polystyrene or an endothelial cell monolayer is required for CRP-induced chemokine secretion in human primary monocytes [14]. The aim of the present study was to test the potential inhibitory effect of statins on CRP-mediated pro-atherosclerotic effects in human adherent monocytes. Statins [also called 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors] are used as lipid-lowering agents for reducing high cholesterol-mediated cardiovascular risk [15, 16]. More recently, the beneficial effects of statins have been extended to a direct immunomodulation of monocyte-mediated inflammatory processes (including chronic inflammatory diseases, such as atherogenesis and RA), which are independent of their effects on cholesterol levels [17–19]. In particular, statins have been shown to reduce MCP-1-induced monocyte recruitment in mice [20] and the expression of prostaglandin E2 receptors in human atherosclerotic plaques and monocytic cells [21]. Macrophage cyclo-oxygenase-2 (COX-2) expression, which plays a pivotal role in inflammatory mediator synthesis, has also been shown to be modulated by statins [22]. Furthermore, statins have been shown to be bone protective and reverse endothelial dysfunction in arthritis models [23–25]. Finally, direct anti-inflammatory properties of statins have been shown in cells from synovium of RA patients [26, 27]. On the basis of these evidences, we aimed to compare the effect of two different statins (simvastatin or atorvastatin) on adherent monocytes in the presence of increased concentrations of CRP. A possible modulating effect of statins on CRP-mediated chemokine secretion, adhesion molecule expression as well as migration was investigated, together with the intracellular signalling pathways involved.

Materials and methods

Reagents

Recombinant human CRP was from R&D Systems Europe (Abingdon, UK). As reported by the manufacturer, the purity of the compound was >97% (determined by SDS–PAGE and visualized by silver stain) and endotoxin level was <1.0 EU/1 μg of CRP [determined by the Limulus amoebocyte lysate (LAL) method]. Simvastatin and atorvastatin were obtained from a commercial source and dissolved as previously described [28]. Appropriate dilutions of vehicle (ethanol) were added as control in all experiments. L-Mevalonate, farnesylpyrophosphate (FPP) and geranylgeranyl-pyrophosphate (GGPP) were from Sigma-Aldrich (St Louis, MI, USA). RPMI 1640 medium was from Gibco (Grand Island, NY, USA). BSA was from Sigma-Aldrich. Recombinant human CCL2, anti-phosphorylated extracellular signal-regulated kinase (ERK) 1/2 (T202/Y204, AF1018)

[1]Division of Cardiology, Geneva University Hospital, Faculty of Medicine, Foundation for Medical Researches, Geneva, Switzerland.

Submitted 18 August 2008; revised version accepted 19 November 2008.

Correspondence to: F. Montecucco, Cardiology Division, Geneva University Hospital, Faculty of Medicine, 64 Avenue Roseraie, 1211 Geneva, Switzerland.
E-mail: fabrizio.montecucco@medecine.unige.ch

polyclonal antibody (Ab), anti-phosphorylated p38 mitogen-activated protein kinase (MAPK) (T180/Y182, AF869) polyclonal Ab and anti-ERK 1/2 (AF1576) Polyclonal Ab were all purchased from R&D Systems Europe. Phycoerythrin (PE)-conjugated anti-human CD54/intercellular adhesion molecule (ICAM)-1 Ab (No. 555511) and fluorescein isothiocyanate (FITC)-conjugated anti-human CD14 Ab (No. 555397) were obtained from BD Pharmingen (Allschwil, CH, Switzerland). Anti-p38 MAPK (H-147, sc-7149) polyclonal Ab, anti-Akt1/2/3 (H-136, sc-8312) polyclonal Ab and anti-phopshorylated Akt1/2/3 (Thr^{308}, sc-16646-R) polyclonal Ab were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The kinase inhibitor LY-294002 [phosphatidylinositol 3-kinase (PI3K) inhibitor] was obtained from Sigma-Aldrich and PD-98059 [mitogen-activated protein kinase (MEK) inhibitor] and SB-203580 (p38 MAPK inhibitor) were obtained from BioMol Research Laboratories (Plymouth Meeting, PA, USA). 1L-6-hydroxymethyl-chiro-inositol 2[(R)-2-*O*-methyl-3-octadecyl chromate] (Akt inhibitor) was from Calbiochem (San Diego, CA, USA).

Isolation and culture of human primary monocytes

Human monocytes were isolated from buffy coats of healthy volunteers without clinical signs of inflammatory disorders, after informed consent, as previously described [29]. The protocol and study were approved by the local ethics committee and they conformed with the principles outlined in the Declaration of Helsinki. Briefly, after centrifugation on Ficoll–Hypaque density gradient, mononuclear cells were collected from the interface and washed with 0.9% sodium chloride. Then, monocytes were purified from the upper interface of a hypotonic Percoll density gradient. Purity of monocytes was determined by flow cytometric analysis (using anti-human CD14 Ab from BD Pharmingen), confirming that at least 85% purity was achieved in all experiments. Cells were cultured in adherence to polystyrene dishes at a concentration of 5×10^6 cells/ml in serum-free RPMI 1640 medium containing 25 mmol/l HEPES and 500 ng/ml polymyxin B (Sigma-Aldrich) at 37°C in a humidified atmosphere with 5% CO_2, unless otherwise specified [14].

Chemokine secretion assay

Monocytes were cultured in the presence or absence of 10 μg/ml CRP for 12 h [14]. For statin dose–response experiments, monocytes were pre-treated for 2 h in the presence of different concentrations (0, 0.01, 0.1 and 1 μM) of simvastatin or atorvastatin. In other experiments, monocytes were pre-treated with kinase inhibitors, LY294002 (50 μM, PI3K inhibitor), Akt inhibitor (40 μM), PD98059 (10 μM, MEK 1 inhibitor) or SB203580 (1 μM, p38 MAPK inhibitor) for 2 h before adding CRP. The concentrations of kinase inhibitors used in these experiments were selected on the basis of previous dose–response experiments (data not shown). In selective experiments, monocytes were pre-treated with 1 μM simvastatin or 1 μM atorvastatin in the presence or absence of 100 μM mevalonate, 10 μM GGPP or 10 μM FPP for 2 h before adding CRP [30]. CCL2, CCL3 and CCL4 levels were measured in supernatants of monocyte cultures by using ELISA kits (R&D Systems Europe). ELISA assays were performed by following the manufacturer's instructions.

Flow cytometry

Monocytes were cultured in the presence or absence of 10 μg/ml CRP for 30 min (CD11b and CD18 analysis), 24 h (ICAM-1 analysis) or 2 h, 12 h and 24 h (CD32 and CD64 analysis), respectively. In selective experiments, monocytes were pre-incubated for 2 h in the presence or absence of different concentrations (0.01, 0.1 and 1 μM) of simvastatin, atorvastatin or kinase inhibitors LY294002 (50 μM), Akt inhibitor (40 μM), PD98059 (10 μM) or SB203580 (1 μM) and then co-incubated with CRP. The concentrations of kinase inhibitors used in these experiments were selected on the basis of previous dose–response experiments (data not shown). In additional experiments, monocytes were pre-treated with 1 μM simvastatin or 1 μM atorvastatin in the presence or absence of 100 μM mevalonate, 10 μM GGPP or 10 μM FPP for 2 h and then co-incubated with CRP. Then, culture supernatants were removed and cells washed with PBS to remove non-adherent cells. Adherent monocytes were collected by scraping with a plastic policeman (Costar Cambridge, USA) and pipetting energetically. Then the cells were stained with FITC- or PE-labelled antibodies to anti-human CD11b, CD18 (R&D Systems Europe Ltd), CD54, CD32 and CD64 (BD Pharmingen^{TM}), as well as corresponding isotype controls. CellQuest software was used for acquisition and analysis on a FACSCalibur (BD Biosciences, Heidelberg, Germany). Data were expressed as mean fluorescence intensities (MFIs), compared with baseline expression (defined as 100%).

Cytotoxicity assay

Cell death was determined by quantification of lactate dehydrogenase (LDH) release in cell culture supernatants of adherent and suspension cultures after 12 and 24 h (BioVision, Mountain View, CA, USA).

Modified Boyden chamber migration assay

Isolated monocytes were washed three times with chemotaxis medium (RPMI containing 25 mmol/l HEPES and 1% BSA; Sigma-Aldrich) [29]. Monocytes were pre-incubated for 2 h in the presence or absence of different concentrations (0.01, 0.1 and 1 μM) of simvastatin, atorvastatin or kinase inhibitors LY294002 (50 μM), Akt inhibitor (40 μM), PD98059 (10 μM), SB203580 (1 μM) and then tested for migration in response to control medium (CTL) or 40 μg/ml CRP [31]. The concentrations of kinase inhibitors used in these experiments were selected on the basis of previous dose–response experiments (data not shown). In additional experiments, monocytes were pre-treated with 1 μM simvastatin or 1 μM atorvastatin in the presence or absence of 100 μM mevalonate, 10 μM GGPP or 10 μM FPP for 2 h and then tested for migration in response to control medium (CTL) or 40 μg/ml CRP. Monocyte chemotaxis was assessed in a 48-well microchemotaxis modified Boyden chamber (Neuro Probe, Gaithersburg, MD, USA) using a 5 μm pore size, 5-μm thick polyvinylpyrrolidone-free polycarbonate filter (Neuro Probe). Cells were seeded in upper wells, while medium or chemoattractant solutions were added to the lower wells. The chamber was incubated for 60 min at 37°C in a humidified atmosphere with 5% CO_2. Then, filters were removed from the chambers and stained with Diff-Quick (Baxter, Rome, Italy). Cells in five random oil-immersion fields were counted at 1000× magnification (blinded observer) and the chemotaxis index was calculated from the number of cells migrated to the chemokine divided by the number of cells migrated to the medium.

Western blot analysis

Monocytes were cultured at a concentration of 5×10^6 cells/ml in serum-free RPMI medium containing 25 mM HEPES with control medium alone, 10 μg/ml CRP for different time points (1, 5, 10, 15 or 30 min) or 10 nM r-CCL2 (positive control: 15 min of incubation for activating ERK 1/2 or 5 min of incubation for activating p38 MAPK or Akt) [29]. The reaction was stopped on ice and cells were centrifuged at 4°C to remove culture supernatants. Total protein was extracted in lysis buffer containing 20 mM Tris–HCL (pH 7.5), 150 mM NaCl, 10 mM NaF, 1% Nonidet P-40, 10 μg/ml glycerol, 1 mM phenylmethanesulphonylfluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin and 0.5 mM Na_3VO_4. Equal amounts of protein (40 μg) for each sample were electrophoresed through polyacrylamide–SDS gels

and transferred by electroblotting onto nitrocellulose membranes. Membranes were blocked for 1 h in 5% (wt/vol) non-fat dry milk before being incubated with appropriate dilutions of anti-phospho-ERK 1/2, anti-phospho-Akt or anti-phospho-p38 MAPK primary Abs as well as corresponding secondary Abs. Blots were developed using the ECL system (Immobilon Western, Millipore, USA). Membranes were then stripped, reblocked and reprobed to detect total ERK 1/2, Akt or p38 MAPK. Immunblots were scanned and quantification was carried out by Image Quant software version 3.3 (Molecular Dynamics, Sunnyvale, USA). Values were normalized to total amounts of ERK 1/2, Akt or p38 MAPK, respectively, and expressed as percentages of medium control (defined as 100%).

Statistical analysis

All data were expressed as mean ± S.E.M. One-way analysis of variance with Bonferroni's post-test was performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego, CA, USA). Differences with P-values <0.05 were considered statistically significant.

Results

Statins inhibit CRP-induced secretion of the chemokines CCL2, CCL3 and CCL4 in human adherent monocytes

To study a possible modulation of CRP-mediated chemokine secretion, we treated adherent monocytes with increasing concentrations of simvastatin or atorvastatin. The incubation of adherent monocytes with 10 μg/ml CRP resulted in a significant induction of CCL2 secretion as compared with untreated cells (Fig. 1A). Simvastatin (0.3–3 μM) and atorvastatin (1 μM) significantly reduced CRP-mediated secretion of CCL2 (Fig. 1A). Similarly, simvastatin and atorvastatin dose-dependently reduced CCL3 and CCL4 secretion in human adherent monocytes (Fig. 1B and C). No inhibitory effect on CRP-induced chemokine secretion was observed in the presence of appropriate dilutions of ethanol (statin vehicle), indicating that the inhibition of CRP-induced chemokine secretion was indeed mediated by statins and not due to a contamination of the vehicle (data not shown). In none of the experimental conditions did we observe an increased cytotoxicity, as determined by release of LDH (data not shown).

Statins inhibit CRP-induced ICAM-1, but not CD11b, CD18 up-regulation in adherent human monocytes

We next investigated the effect of statins on CRP-induced CD11b, CD18 and ICAM-1 up-regulation in adherent human monocytes. Treatments with 1 μM simvastatin or atorvastatin (Fig. 2A and D) significantly inhibited CRP-mediated ICAM-1 up-regulation. No significant inhibition of CRP-induced CD11b or CD18 up-regulation was observed in the presence of different concentrations of simvastatin or atorvastatin (Fig. 2B–D). In all experiments, appropriate dilutions of ethanol (statin vehicle) did not induce any effect (data not shown).

Statins inhibit human monocyte migration in response to CRP

The ability of CRP to induce monocyte migration has been previously described [31]. On the basis of this previous study, we selected 40 μg/ml as an effective concentration to test monocyte locomotion in the presence of statins. Both simvastatin and atorvastatin at 1 μM significantly inhibited monocyte migration in response to CRP (Fig. 3A and B).

Statins do not influence CD32 or CD64 (CRP receptors) expression on adherent human monocyte surface

In order to investigate the molecular mechanisms by which statins reduced CRP-mediated chemokine secretion, ICAM-1 up-regulation and chemotaxis, we investigated if treatment with simvastatin or atorvastatin could modulate the expression levels of CD32 and CD64 (CRP receptors) [14, 32]. Co-incubation of CRP-treated monocytes with 1 μM simvastatin or 1 μM atorvastatin did not modify CD32 or CD64 expression in comparison with control medium (CTL), as tested at different time points (see supplementary figure IA and B available as supplementary data at *Rheumatology* Online). Supplementary figure IC and D (available as supplementary data at *Rheumatology* Online) shows that CD32 or CD64 expression was not modulated by either co-incubation (12 h) of CRP-treated monocytes with different concentrations of statins nor incubation (12 h) of untreated monocytes with 1 μM simvastatin or 1 μM atorvastatin.

CRP-induced chemokine secretion, ICAM-1 up-regulation and chemotaxis is dependent on ERK 1/2 activation

Given the lack of statin-mediated effect on CRP receptor surface expression, we next investigated their possible inhibitory activity on intracellular pathways. We first investigated a possible CRP-mediated activation of different signalling pathways in human primary monocytes. Previous studies have shown that CRP induces mitogen-activated protein kinase (MAPK) phosphorylation in human monocytes and monocytic cell lines [33–36].

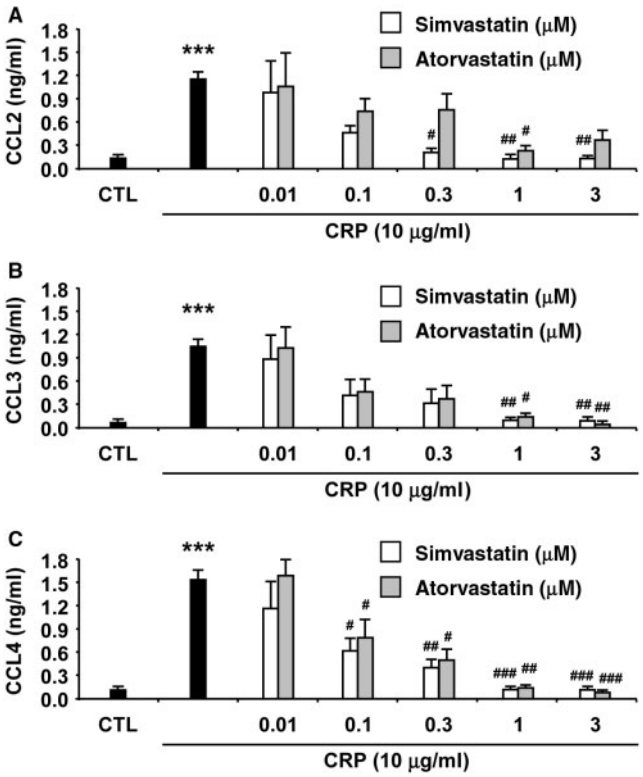

FIG. 1. Statins reduce CRP-induced CCL2, CCL3 and CCL4 secretion in adherent human monocytes. Effect of simvastain and atorvastatin in chemokine secretion in CRP-treated monocytes cultured in adherence to polystyrene dishes for 12 h (data are expressed as mean ± S.E.M.). (**A**) CCL2: CTL and CRP alone: black bars, $n=24$, $^{***}P<0.001$ *vs* CTL; simvastatin: white bars, $n=18$, $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* CRP; atorvastatin: grey bars, $n=16$, $^{\#}P<0.05$ *vs* CRP. (**B**), CCL3: CTL and CRP alone: black bars, $n=24$, $^{***}P<0.001$ *vs* CTL; simvastatin: white bars, $n=18$, $^{\#\#}P<0.01$ *vs* CRP; atorvastatin: grey bars, $n=16$, $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* CRP). (**C**) CCL4: CTL and CRP alone: black bars, $n=16$, $^{***}P<0.001$ *vs* CTL; simvastatin: white bars, $n=12$, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs* CRP; atorvastatin: grey bars, $n=10$, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs* CRP.

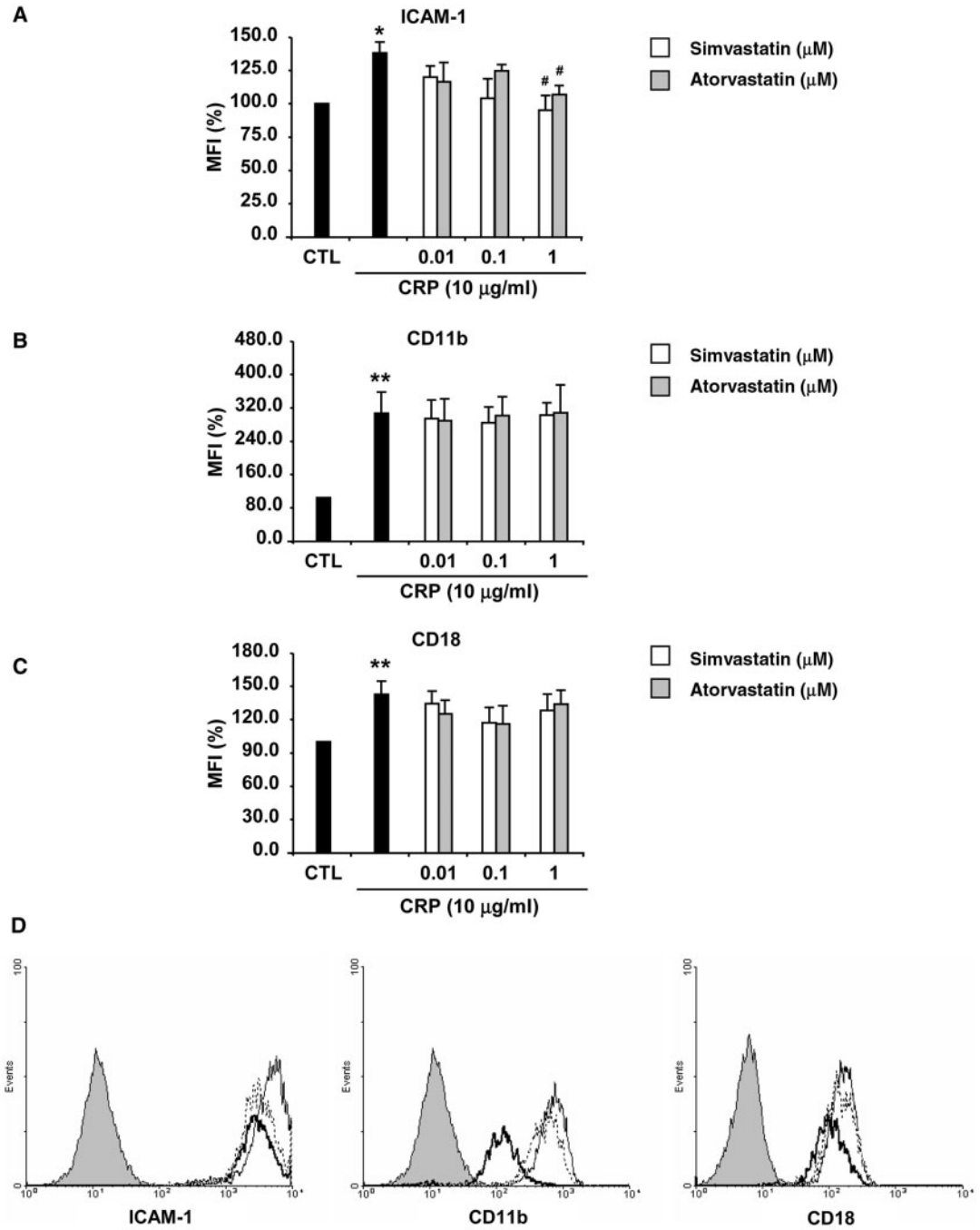

FIG. 2. Statins reduce CRP-induced ICAM-1 up-regulation in adherent human monocytes. Effect of simvastatin and atorvastatin on adhesion molecule expression in adherent monocytes in response to 10 μg/ml CRP. (**A**) ICAM-1 ($n=10$, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP). (**B**) CD11b ($n=7$, $^{**}P<0.01$ *vs* CTL). (**C**) CD18 ($n=8$, $^{**}P<0.01$ *vs* CTL). (**D**) Representative flow cytometric analyses of ICAM-1, CD11b and CD18 expression on adherent monocytes. As 1 μM atorvastatin or 1 μM simvastatin induce similar effects in CRP-treated monocytes, only simvastatin data are shown in representative histograms. The respective histograms show isotype control (solid fill in grey) and staining of untreated (bold black line), CRP-treated (black line, unfilled) or CRP-treated in the presence of 1 μM simvastatin monocytes (dotted line).

Therefore, we focused our investigation on MAPK. A 10 μM PD98059 (inhibitor of MEK 1, a kinase directly activating ERK 1) inhibited CRP-induced CCL2, CCL3 and CCL4 secretion (Fig. 4A–C). PD98059 also significantly inhibited CRP-induced ICAM-1 up-regulation and migration (Fig. 4D and E). The inhibitors of PI3K/Akt and p38 MAPK did not induce any effect in CRP-mediated chemokine secretion, ICAM-1 up-regulation or migration (Fig. 4A–E). A 15–30 min of exposure to 10 μg/ml CRP increased ERK 1/2 phosphorylation, in a manner comparable with CCL2 (positive control). Fifteen minutes of incubation in the presence of 10 μg/ml CRP also induced Akt phosphorylation, while a non-specific increase of p38 MAPK activation was observed after 5–15 min (Fig. 5A). Densitometric analysis of different experiments confirmed these data (Fig. 5B–E).

Statins reduce CRP-induced chemokine secretion, ICAM-1 up-regulation and chemotaxis through the inhibition of HMG-CoA reductase-ERK 1/2 pathway

After having identified the signalling pathway in CRP-mediated pro-inflammatory activities, we investigated their possible modulation by statins. In particular, we focused on statin-induced inhibition of HMG-CoA reductase and the downstream kinase activation pattern. Simvastatin- and atorvastatin-mediated

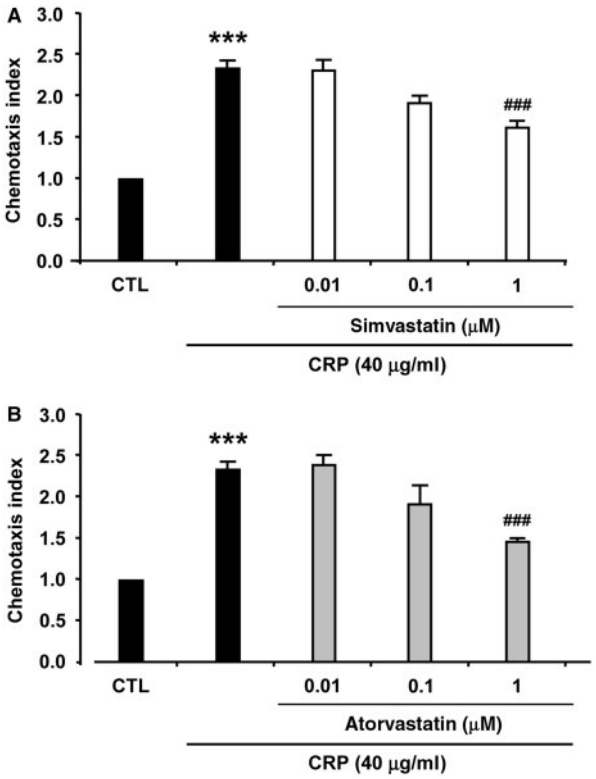

FIG. 3. Statins reduce human monocyte migration in response to CRP. (**A**) Effect of simvastatin on CRP-induced monocyte migration ($n=6$, $^{***}P<0.001$ vs CTL, $^{###}P<0.001$ *vs* CRP). (**B**) Effect of atorvastatin on CRP-induced monocyte migration ($n=6$, $^{***}P<0.001$ *vs* CTL, $^{###}P<0.001$ *vs* CRP).

inhibition of CCL2, CCL3 and CCL4 in the presence of CRP was reversed by the co-incubation with L-mevalonate or farnesylpyrophosphate (Fig. 6A–C), indicating that CRP-induced chemokine secretion was dependent on the activation of these two mediators. Co-incubation with L-mevalonate or farnesylpyrophosphate also reversed statin-induced inhibition of CRP-mediated ICAM-1 expression or migration on human monocytes (Fig. 6D and E). On the other hand, geranylgeranyl-pyrophosphate did not induce any effect on statin-mediated activities (Fig. 6A–E). In accordance with these data, simvastatin or atorvastatin inhibited CRP-induced ERK 1/2 phosphorylation (Fig. 7A and C). Co-incubation with L-mevalonate or farnesylpyrophosphate, but not geranylgeranyl-pyrophosphate significantly reversed the statin-induced inhibition of CRP-mediated ERK 1/2 phosphorylation, as shown by western blot analysis and densitometries (Fig. 7A and B). These data confirm that statin-induced inhibition of the HMG-CoA reductase-ERK 1/2 pathway is necessary to reduce CRP-mediated chemokine secretion, ICAM-1 up-regulation and migration in human primary monocytes (Fig. 8A–D).

Discussion

Monocytes are inflammatory cells which play a crucial role in various chronic inflammatory disorders, such as atherosclerosis and RA. These cells sustain inflammatory processes through the secretion of several soluble mediators. Although a great number of chemokines and cytokines have been already identified, much remains to be discovered. Under certain conditions, such as adherence to endothelial cells or other substrates, CRP-treated monocytes secrete high concentrations of CC chemokines, such as CCL2, CCL3 and CCL4 [14]. These chemokines contribute to inflammation through a double activity. First, they induce the recruitment of other inflammatory cells from the blood stream

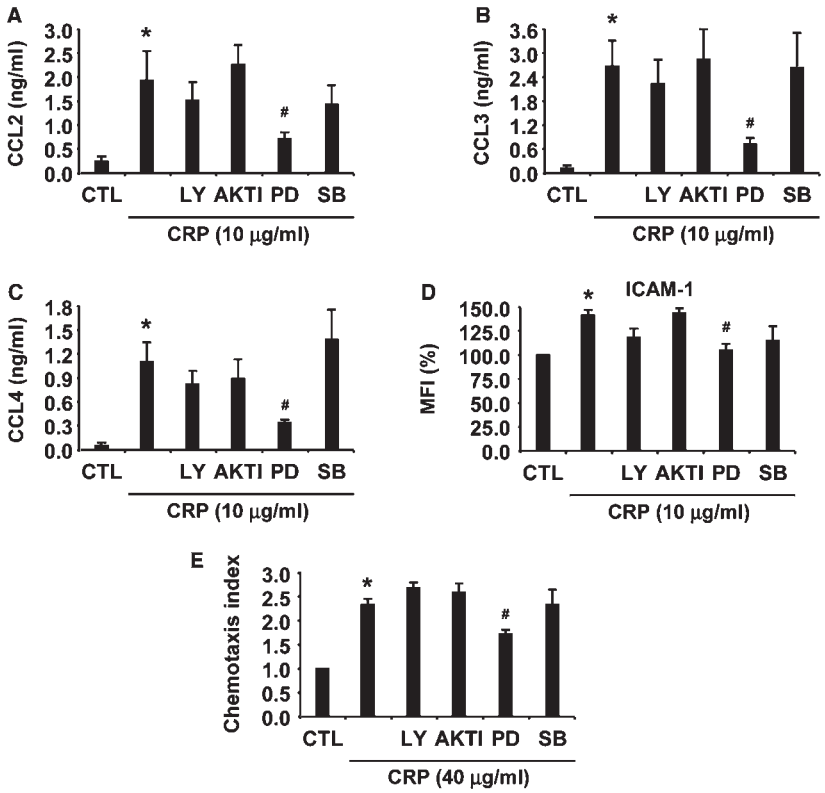

FIG. 4. Effect of kinase inhibitors on CRP-induced chemokine secretion, ICAM-1 up-regulation and migration. Effect of 50 μM LY294002 (LY, PI3K inhibitor), 40 μM Akt inhibitor (AKTI), 10 μM PD98059 (PD, MEK 1 inhibitor), 1 μM SB203580 (SB, p38 MAPK inhibitor) on CRP-mediated pro-inflammatory effects. (**A**) CCL2 secretion ($n=8$ for CTL, CRP alone, CRP plus LY, CRP plus AKTI, CRP plus SB, $n=6$ for CRP plus PD, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP). (**B**) CCL3 secretion ($n=10$ for CTL, CRP alone, CRP plus LY, CRP plus AKTI, CRP plus SB, $n=6$ for CRP plus PD, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP). (**C**) CCL4 secretion ($n=12$ for CTL, CRP alone, CRP plus LY, CRP plus AKTI, CRP plus SB, $n=6$ for CRP plus PD, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP). (**D**) ICAM-1 expression ($n=8$ for CTL, CRP alone, CRP plus LY, CRP plus AKTI, CRP plus SB, $n=6$ for CRP plus PD, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP). (**E**) Monocyte migration in response to CRP ($n=6$, $^{*}P<0.05$ *vs* CTL, $^{\#}P<0.05$ *vs* CRP).

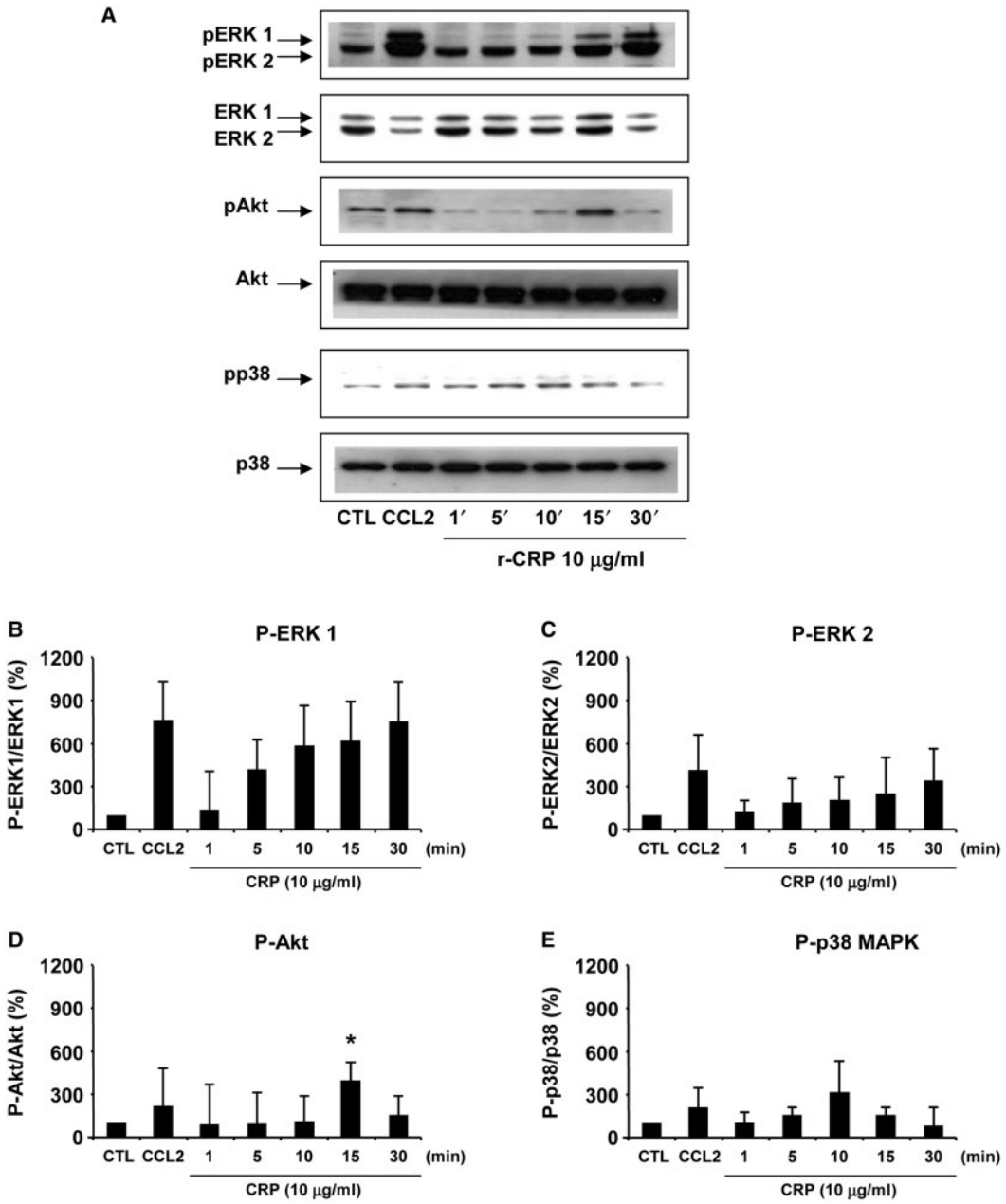

FIG. 5. CRP-induced ERK 1/2, but not PI3K/Akt or p38 MAPK signalling pathways are involved in the modulation of chemokine secretion, ICAM-1 up-regulation and migration. Monocytes were cultured in adherence to polystyrene in the presence of medium alone (CTL) or CRP 10 μg/ml for the indicated times or 10 nM CCL2 (15 min for ERK 1/2 or 5 min for Akt and p38 MAPK, as positive controls) and analysed by western blot to detect phosphorylated (P) and total ERK 1/2, Akt and p38 MAPK. (**A**) Representative western blots of different experiments ($n=2$ for ERK 1/2 and $n=3$ for Akt and p38 MAPK). (**B**–**E**), Quantification of densitometries of kinase activation of different experiments (data are expressed as mean ± S.E.M., $^{*}P<0.05$ *vs* CTL).

within the inflamed tissues. Second, they reduce the expression of their cognate receptors on monocyte surface membrane, thus favouring leucocyte retention and differentiation within inflamed tissues [14]. CRP is a crucial player, which contributes to the establishment of this deleterious vicious circle. In this study, we tested the ability of two statins (simvastatin and atorvastatin) to inhibit pro-inflammatory effects of CRP in adherent monocytes. Importantly, the effective concentrations of statins and CRP used in the present experiments are within the range of human serum levels detected in patients with cardiovascular disease under statin treatment [7, 37]. The major finding of this study was the dose-dependent inhibition induced by both simvastatin and atorvastatin on CRP-mediated CCL2, CCL3 and CCL4 secretion, ICAM-1 up-regulation and migration in human primary monocytes. No effect was observed on CRP-induced expression of CD11b and CD18. These adhesion molecules, which form the Mac-1 complex on the cell surface, have been identified as crucial players in CRP-induced chemokine secretion in human adherent monocytes [14]. The absence of inhibitory activity of statins on CRP-induced Mac-1 up-regulation indicates that both simvastatin and atorvastatin selectively inhibit CRP-mediated intracellular signalling rather than co-stimulatory activation through Mac-1/ICAM-1 interaction. In order to investigate the inhibitory molecular mechanism triggered by statins, we focused on the possible modulation by these molecules of CRP receptors and CRP post-receptor pathways. Statins are selective competitive inhibitors of

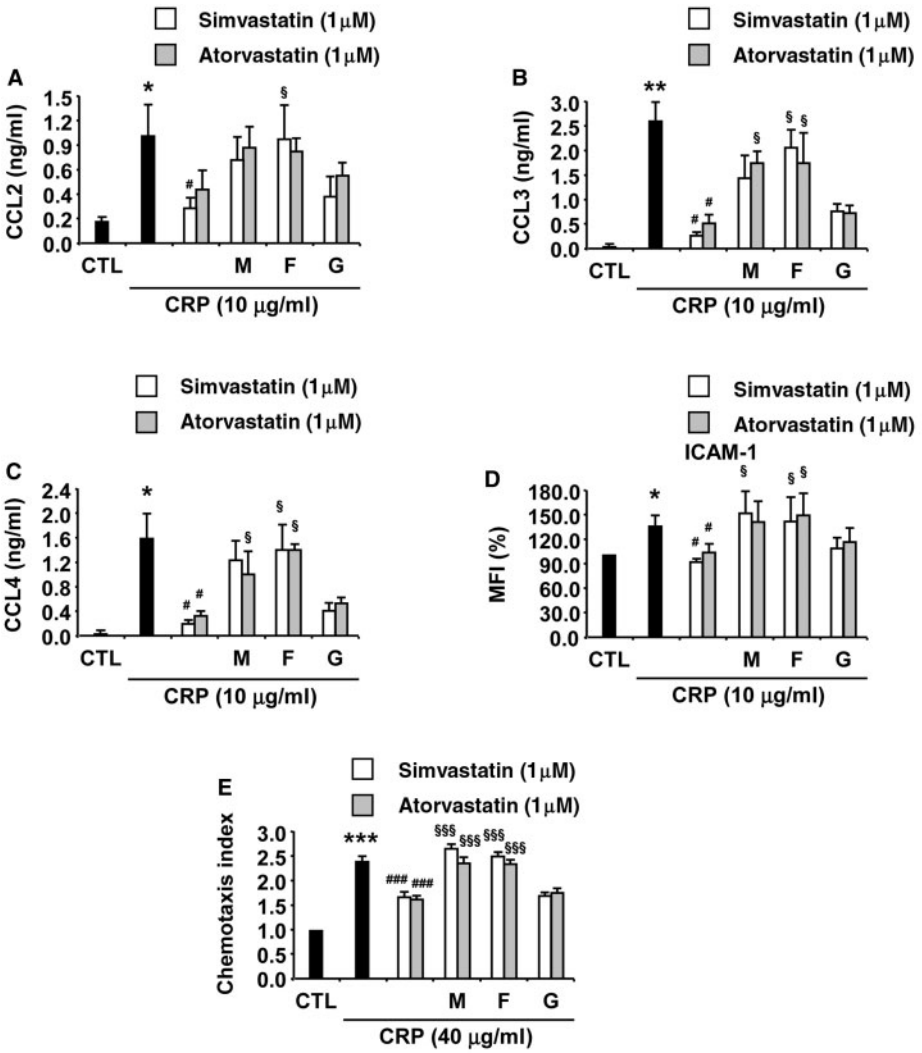

FIG. 6. Statins reduce CRP-induced chemokine secretion, ICAM-1 up-regulation and migration through the inhibition of HMG-CoA reductase. Monocytes were pre-incubated with 100 μM L-mevalonate (M), 10 μM (F), 10 μM (G) and 1 μM simvastatin or 1 μM atorvastatin, and then stimulated with CRP. (**A**) CCL2 secretion (n = 9, $^{*}P < 0.05$ *vs* CTL, $^{\#}P < 0.05$ *vs* CRP, $^{\S}P < 0.05$ *vs* simvastatin or atorvastatin alone). (**B**) CCL3 secretion ($n = 7$, $^{**}P < 0.01$ *vs* CTL, $^{\#}P < 0.05$ *vs* CRP, $^{\S}P < 0.05$ *vs* simvastatin or atorvastatin alone). (**C**) CCL4 secretion ($n = 6$, $^{*}P < 0.05$ *vs* CTL, $^{\#}P < 0.05$ *vs* CRP, $^{\S}P < 0.05$ *vs* simvastatin or atorvastatin alone). (**D**) ICAM-1 expression ($n = 6$, $^{*}P < 0.05$ *vs* CTL, $^{\#}P < 0.05$ *vs* CRP, $^{\S}P < 0.05$ *vs* simvastatin or atorvastatin alone). (**E**) Monocyte migration in response to CRP ($n = 6$, $^{***}P < 0.001$ *vs* CTL, $^{\#\#\#}P < 0.001$ *vs* CRP, $^{\S\S\S}P < 0.001$ *vs* simvastatin or atorvastatin alone).

HMG-CoA reductases, the rate-limiting enzyme in the synthesis of cholesterol [38]. However, this enzyme has been recently shown to exhibit crucial immunomodulatory activities in inflammatory cells [20, 39–46]. The majority of the studies showed anti-oxidant, anti-inflammatory, anti-thrombotic and anti-atherogenic effects of statins [47–49]. On the contrary, statin-induced promotion of pro-inflammatory responses has also been shown [43, 44]. Statins modulate inflammatory cell functions mainly through the regulation of intracellular kinase pathways downstream to HMG-CoA reductase [33, 36, 50–54]. However, functional differences among members of the statin family suggest that these pharmacological molecules could also directly modulate inflammatory pathways, independently of the inhibition of HMG-CoA reductase [55–57]. In the present work, we show that the inhibition of HMG-CoA reductase by statins results in the downstream inhibition of ERK 1/2 phosphorylation. This is supported by three different findings. First, ERK 1/2 phosphorylation is crucial for CRP-mediated pro-inflammatory activities in human monocytes, as shown by the experiments with PD98059 (the selective inhibitor of MEK 1, a kinase which directly activated ERK 1) and the phosphorylation of ERK 1/2 induced by CRP. The involvement of ERK 1/2 in CRP signalling has already been shown in monocytic cell lines and also in human peripheral blood monocytes [33, 35]. We confirmed the CRP-induced phosphorylation and we provide evidence for the involvement of ERK 1/2 in CRP-mediated CCL2, CCL3 and CCL4 secretion, ICAM-1 up-regulation and migration. Second, co-incubation with L-mevalonate or FPP significantly reversed the statin effect on chemokine secretion, ICAM-1 up-regulation, migration and ERK 1/2 phosphorylation, suggesting that simvastatin and atorvastatin blocked CRP-induced pro-inflammatory activity in human monocytes through the direct inhibition of the HMG-CoA reductase-FPP-ERK 1/2 pathway. Third, statin did not down-regulate the surface expression of CRP receptors. This is a novel and important finding in primary human monocytes. The lack of activity of a member of the statin family (fluvastatin) on CD32 and CD64 expression has been previously described in U937 cells (a human leukaemic monocyte lymphoma cell line) and further supports the modulation of signalling events downstream of receptor activation [58]. On the other hand, statins have been shown to modulate the expression of chemokine

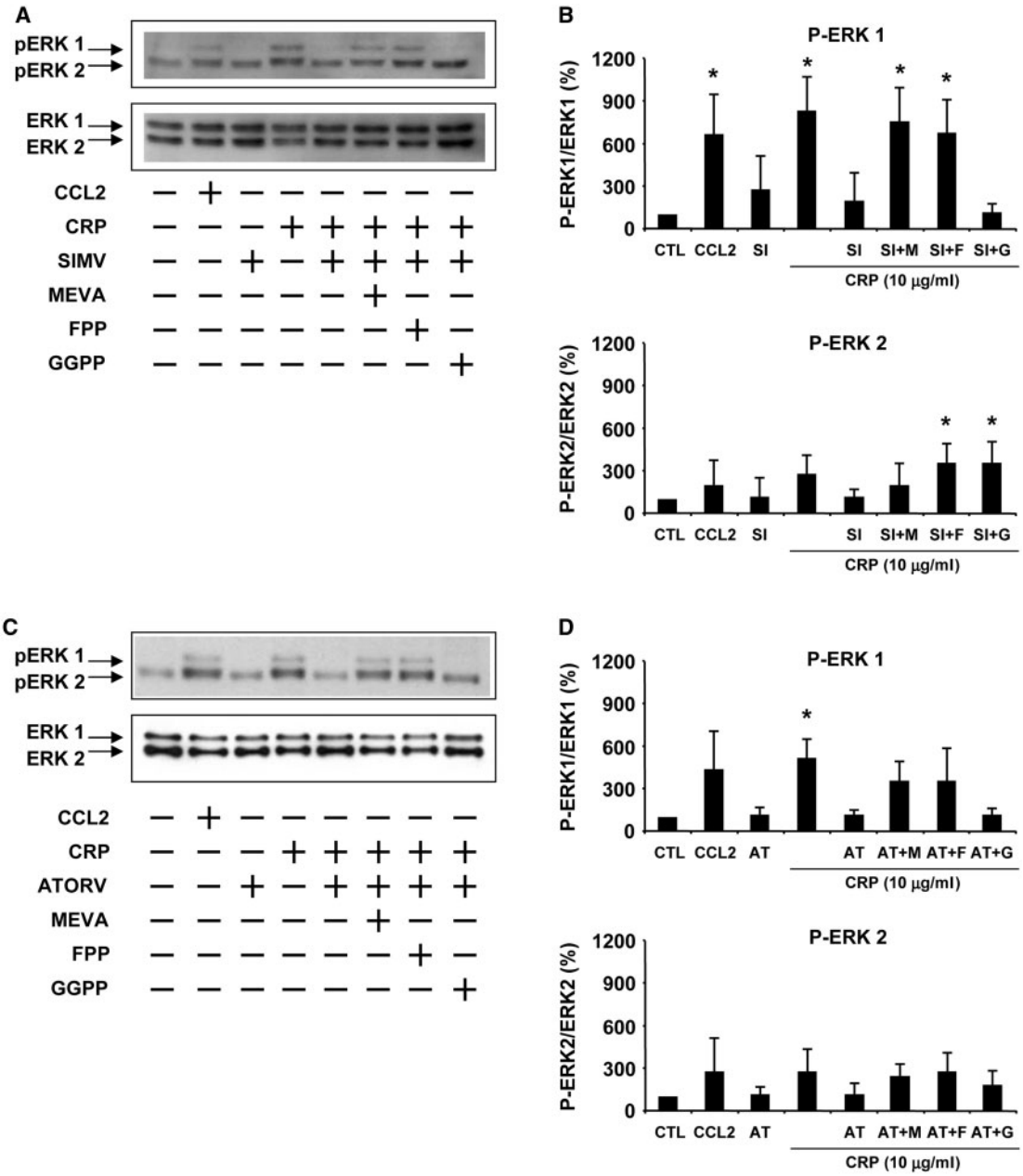

FIG. 7. Statins reduce CRP-induced chemokine secretion, ICAM-1 up-regulation and migration through the inhibition of HMG-CoA reductase-ERK1/2 pathway. (**A**) Representative western blot of ERK 1/2 activation in the presence or absence of 10 nM CCL2 (15 min, positive control), 10 μg/ml CRP (30 min), 1 μM simvastatin (2 h, SIMV), 100 μM L-mevalonate (2 h, MEVA), 10 μM farnesyl (2 h, FPP), 10 μM geranyl (2 h, GGPP). (**B**) Quantification of densitometries of ERK 1 and ERK 2 activation of different experiments: CTL (control); CCL2 (positive control); SI (simvastatin); SI+M (simvastatin + L-mevalonate); SI+F (simvastatin + FPP); SI+G (simvastatin + GGPP). Data are expressed as mean ± S.E.M., $n=4$, $^*P<0.05$ *vs* CTL. (**C**) Representative western blot of ERK 1/2 activation in the presence or absence of 10 nM CCL2 (15 min, positive control), 10 μg/ml CRP (30 min), 1 μM atorvastatin (2 h, ATORV), 100 μM L-mevalonate (2 h, MEVA), 10 μM farnesyl (2 h, FPP), 10 μM geranyl (2 h, GGPP). (**D**) Quantification of densitometries of ERK 1 and ERK 2 activation of different experiments: CTL (control); CCL2 (positive control); AT (atorvastatin); AT+M (atorvastatin + L-mevalonate); AT+F (atorvastatin + FPP); AT+G (atorvastatin + GGPP). Data are expressed as mean ± S.E.M., $n=3$, $^*P<0.05$ *vs* CTL.

receptors and adhesion molecules in human monocyte/macrophages [30, 46, 59]. Moreover, toll-like receptor 4, angiotensin II type 1 receptor (AT_1R) and CD36 expression are modulated by statins in human monocytes [60–62]. In addition, statins also reduced the expression of CD40, CD83 and CD86 in human monocyte-derived dendritic cell maturation [63]. In conclusion, although statins are well-known regulators of different receptors on human monocytes, in the present study we showed that statins do not modulate CRP receptor (CD32 and CD64) expression on human primary monocytes.

To summarize, we provide evidence that statins reduce CRP-induced chemokine secretion, ICAM-1 up-regulation and migration in human primary monocytes through the inhibition of the HMG-CoA-ERK 1/2 pathway (Fig. 8). The HMG-CoA-ERK 1/2 pathway could represent a novel and promising target to reduce CRP-mediated pro-inflammatory activities on human monocytes. This study further supports the direct beneficial effects of statins (independently of lowering cholesterol levels) in the modulation of monocyte-mediated inflammatory diseases characterized by increased levels of CRP. Mainly for the inadequate inflammation suppression and the multiple concomitant drug therapy, the reduction of cardiovascular burden in patients with RA is a more complex process than in the general population. For this reason, among various anti-RA therapies which could

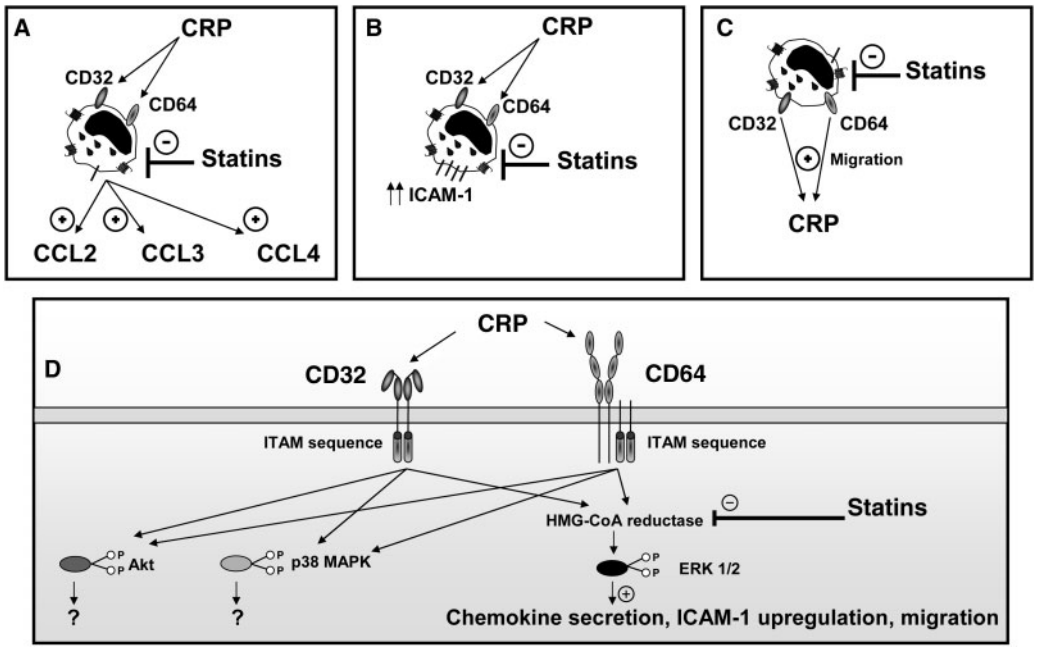

FIG. 8. Statins reduce CRP-mediated pro-inflammatory effects in human monocytes through inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase-ERK1/2 pathway. Simvastatin and atorvastatin reduce CRP-mediated chemokine secretion (**A**), ICAM-1 up-regulation (**B**) and migration (**C**) in human primary monocytes. Statins inhibit CRP-induced pro-inflammatory effect through inhibition of the HMG-CoA reductase-ERK 1/2 pathway (**D**). Conversely, PI3K/Akt, p38 MAPK which are also activated by CRP are not involved in CRP-mediated pro-inflammatory effects (**D**).

modulate atherogenesis [64], statins may represent a pathophysiological approach independent of cholesterol levels for reducing CRP-mediated pro-inflammatory activity on primary monocytes.

Rheumatology key messages
• CRP induces pro-inflammatory activities in human adherent monocytes through ERK 1/2 activation. • Statins inhibit CRP-mediated pro-inflammatory activities through the inhibition of HMG-CoA-ERK 1/2 pathway. • HMG-CoA-ERK 1/2 pathway could represent a promising target to reduce CRP-mediated activities in human monocytes.

Funding: This work was supported by grants from the Swiss National Science Foundation (#310000-116324 to S.S.); (#3200B0-105896/1 to F.M.). The authors belong to the European Vascular Genomics Network (http://www.evgn.org), a Network of Excellence supported by the European Community.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Bacon PA, Stevens RJ, Carruthers DM, Young SP, Kitas GD. Accelerated atherogenesis in autoimmune rheumatic diseases. Autoimmun Rev 2002;1:338–47.
2 Rosamond W, Flegal K, Furie K *et al.* American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25–146.
3 Solomon DH, Karlson EW, Rimm EB *et al.* Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation 2003;107:1303–7.
4 del Rincón ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001;44:2737–45.
5 Chung CP, Oeser A, Avalos I *et al.* Utility of the Framingham risk score to predict the presence of coronary atherosclerosis in patients with rheumatoid arthritis. Arthritis Res Ther 2006;8:R186.
6 Montecucco F, Mach F. Common inflammatory mediators orchestrate pathophysiological processes in rheumatoid arthritis and atherosclerosis. Rheumatology. Advance Access published October 16, 2008, doi:10.1093/rheumatology/ken395.
7 Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension 2004;44:6–11.
8 Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
9 Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363–9.
10 Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 2000;102:2165–8.
11 Pasceri V, Cheng JS, Willerson JT, Yeh, ET. Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 2001;103:2531–4.
12 Ridker PM. High-sensitivity C-reactive protein, inflammation, and cardiovascular risk: from concept to clinical practice to clinical benefit. Am Heart J 2004;148(1 Suppl.):S19–26.
13 Wu J, Stevenson MJ, Brown JM, Grunz EA, Strawn TL, Fay WP. C-reactive protein enhances tissue factor expression by vascular smooth muscle cells: mechanisms and in vivo significance. Arterioscler Thromb Vasc Biol 2008;28:698–704.
14 Montecucco F, Steffens S, Burger F, Pelli G, Monaco C, Mach F. C-reactive protein (CRP) induces chemokine secretion via CD11b/ICAM-1 interaction in human adherent monocytes. J Leukoc Biol 2008;84:1109–19.
15 Veillard NR, Mach F. Statins: the new aspirin? Cell Mol Life Sci 2002;59:1771–86.
16 Ridker PM, Cannon CP, Morrow D *et al.* Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
17 McCarey DW, McInnes IB, Madhok R *et al.* Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 2004;363:2015–21.
18 Mach F. Statins as immunomodulatory agents. Circulation 2004;109:II15–7.
19 Kwak BR, Mulhaupt F, Mach F. Atherosclerosis: anti-inflammatory and immunomodulatory activities of statins. Autoimmun Rev 2003;2:332–8.
20 Han KH, Ryu J, Hong KH *et al.* HMG-CoA reductase inhibition reduces monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated monocyte recruitment in vivo. Circulation 2005;111:1439–47.
21 Gómez-Hernández A, Sánchez-Galán E, Martín-Ventura JL *et al.* Atorvastatin reduces the expression of prostaglandin E2 receptors in human carotid atherosclerotic plaques and monocytic cells: potential implications for plaque stabilization. J Cardiovasc Pharmacol 2006;47:60–9.
22 Habib A, Shamseddeen I, Nasrallah MS *et al.* Modulation of COX-2 expression by statins in human monocytic cells. FASEB J 2007;21:1665–74.
23 Funk JL, Chen J, Downey KJ, Clark RA. Bone protective effect of simvastatin in experimental arthritis. J Rheumatol 2008;35:1083–91.

24 Mäki-Petäjä KM, Booth AD, Hall FC *et al.* Ezetimibe and simvastatin reduce inflammation, disease activity, and aortic stiffness and improve endothelial function in rheumatoid arthritis. J Am Coll Cardiol 2007;50:852–8.
25 Paraskevas KI. Statin treatment for rheumatoid arthritis: a promising novel indication. Clin Rheumatol 2008;27:281–7.
26 Yokota K, Miyoshi F, Miyazaki T *et al.* High concentration simvastatin induces apoptosis in fibroblast-like synoviocytes from patients with rheumatoid arthritis. J Rheumatol 2008;35:193–200.
27 Lazzerini PE, Lorenzini S, Selvi E *et al.* Simvastatin inhibits cytokine production and nuclear factor-kB activation in interleukin 1beta-stimulated synoviocytes from rheumatoid arthritis patients. Clin Exp Rheumatol 2007;25:696–700.
28 Arnaud C, Burger F, Steffens S *et al.* Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes: new evidence for direct anti-inflammatory effects of statins. Arterioscler Thromb Vasc Biol 2005;25:1231–6.
29 Montecucco F, Burger F, Mach F, Steffens S. CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. Am J Physiol Heart Circ Physiol 2008;294:H1145–55.
30 Hu WL, Qian SB, Li JJ. Decreased C-reactive protein-induced resistin production in human monocytes by simvastatin. Cytokine 2007;40:201–6.
31 Torzewski M, Rist C, Mortensen RF *et al.* C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. Arterioscler Thromb Vasc Biol 2000;20:2094–9.
32 Han KH, Hong KH, Park JH *et al.* C-reactive protein promotes monocyte chemoattractant protein-1-mediated chemotaxis through upregulating CC chemokine receptor 2 expression in human monocytes. Circulation 2004;109:2566–71.
33 Bello G, Cailotto F, Hanriot D *et al.* C-reactive protein (CRP) increases VEGF-A expression in monocytic cells via a PI3-kinase and ERK 1/2 signaling dependent pathway. Atherosclerosis 2008;200:286–93.
34 Lim MY, Wang H, Kapoun AM *et al.* p38 Inhibition attenuates the pro-inflammatory response to C-reactive protein by human peripheral blood mononuclear cells. J Mol Cell Cardiol 2004;37:1111–4.
35 Xie L, Chang L, Guan Y, Wang X. C-reactive protein augments interleukin-8 secretion in human peripheral blood monocytes. J Cardiovasc Pharmacol 2005;46:690–6.
36 Kawahara K, Biswas KK, Unoshima M *et al.* C-reactive protein induces high-mobility group box-1 protein release through activation of p38MAPK in macrophage RAW264.7 cells. Cardiovasc Pathol 2008;17:129–38.
37 Shitara Y, Sugiyama Y. Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol Ther 2006;112:71–105.
38 Gotto AM Jr. Treating hypercholesterolemia: looking forward. Clin Cardiol 2003;26 (Suppl. 1):I21–8.
39 Urbich C, Dernbach E, Zeiher AM, Dimmeler S. Double-edged role of statins in angiogenesis signaling. Circ Res 2002;90:737–44.
40 Kreuzer J, Bader J, Jahn L, Hautmann M, Kübler W, Von Hodenberg E. Chemotaxis of the monocyte cell line U937: dependence on cholesterol and early mevalonate pathway products. Atherosclerosis 1991;90:203–9.
41 Wong B, Lumma WC, Smith AM, Sisko JT, Wright SD, Cai TQ. Statins suppress THP-1 cell migration and secretion of matrix metalloproteinase 9 by inhibiting geranylgeranylation. J Leukoc Biol 2001;69:959–62.
42 Okopień B, Krysiak R, Kowalski J *et al.* Monocyte release of tumor necrosis factor-alpha and interleukin-1beta in primary type IIa and IIb dyslipidemic patients treated with statins or fibrates. J Cardiovasc Pharmacol 2005;46:377–86.
43 Takahashi HK, Mori S, Iwagaki H, Yoshino T, Tanaka N, Nishibori M. Simvastatin induces interleukin-18 production in human peripheral blood mononuclear cells. Clin Immunol 2005;116:211–6.
44 Coward WR, Marei A, Yang A, Vasa-Nicotera MM, Chow SC. Statin-induced proinflammatory response in mitogen-activated peripheral blood mononuclear cells through the activation of caspase-1 and IL-18 secretion in monocytes. J Immunol 2006;176:5284–92.
45 Park SY, Lee JS, Ko YJ *et al.* Inhibitory effect of simvastatin on the TNF-alpha- and angiotensin II-induced monocyte adhesion to endothelial cells is mediated through the suppression of geranylgeranyl isoprenoid-dependent ROS generation. Arch Pharm Res 2008;31:195–204.
46 Veillard NR, Braunersreuther V, Arnaud C *et al.* Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. Atherosclerosis 2006;188:51–8.
47 Terkeltaub R, Solan J, Barry M Jr, Santoro D, Bokoch GM. Role of the mevalonate pathway of isoprenoid synthesis in IL-8 generation by activated monocytic cells. J Leukoc Biol 1994;55:749–55.
48 Corsini A. Fluvastatin: effects beyond cholesterol lowering. J Cardiovasc Pharmacol Ther 2000;5:161–75.
49 Shovman O, Levy Y, Gilburd B, Shoenfeld Y. Antiinflammatory and immunomodulatory properties of statins. Immunol Res 2002;25:271–85.
50 Hiraoka M, Nitta N, Nagai M, Shimokado K, Yoshida M. MCP-1-induced enhancement of THP-1 adhesion to vascular endothelium was modulated by HMG-CoA reductase inhibitor through RhoA GTPase-, but not ERK1/2-dependent pathway. Life Sci 2004;75:1333–41.
51 Yoshida M, Sawada T, Ishii H *et al.* Hmg-CoA reductase inhibitor modulates monocyte-endothelial cell interaction under physiological flow conditions in vitro: involvement of Rho GTPase-dependent mechanism. Arterioscler Thromb Vasc Biol 2001;21:1165–71.
52 Sakamoto T, Ishibashi T, Sugimoto K *et al.* RhoA-dependent PAI-1 gene expression induced in endothelial cells by monocyte adhesion mediates geranylgeranyl transferase I and $Ca2+$ signaling. Atherosclerosis 2007;193:44–54.
53 Ortego M, Gómez-Hernández A, Vidal C *et al.* HMG-CoA reductase inhibitors reduce I kappa B kinase activity induced by oxidative stress in monocytes and vascular smooth muscle cells. J Cardiovasc Pharmacol 2005;45:468–75.
54 Cordle A, Koenigsknecht-Talboo J, Wilkinson B, Limpert A, Landreth G. Mechanisms of statin-mediated inhibition of small G-protein function. J Biol Chem 2005;280:34202–9.
55 Tanimoto A, Murata Y, Wang KY, Tsutsui M, Kohno K, Sasaguri Y. Monocyte chemoattractant protein-1 expression is enhanced by granulocyte-macrophage colony-stimulating factor via Jak2-Stat5 signaling and inhibited by atorvastatin in human monocytic U937 cells. J Biol Chem 2008;283:4643–51.
56 Patel TR, Corbett SA. Simvastatin suppresses LPS-induced Akt phosphorylation in the human monocyte cell line THP-1. J Surg Res 2004;116:116–20.
57 Turner NA, Midgley L, O'Regan DJ, Porter KE. Comparison of the efficacies of five different statins on inhibition of human saphenous vein smooth muscle cell proliferation and invasion. J Cardiovasc Pharmacol 2007;50:458–61.
58 Hillyard DZ, Jardine AG, McDonald KJ, Cameron AJ. Fluvastatin inhibits raft dependent Fcgamma receptor signalling in human monocytes. Atherosclerosis 2004;172:219–28.
59 Takahashi HK, Mori S, Iwagaki H *et al.* Differential effect of LFA703, pravastatin, and fluvastatin on production of IL-18 and expression of ICAM-1 and CD40 in human monocytes. J Leukoc Biol 2005;77:400–7.
60 Marino F, Guasti L, Cosentino M *et al.* Simvastatin treatment in subjects at high cardiovascular risk modulates AT1R expression on circulating monocytes and T lymphocytes. J Hypertens 2008;26:1147–55.
61 Methe H, Kim JO, Kofler S, Nabauer M, Weis M. Statins decrease Toll-like receptor 4 expression and downstream signaling in human $CD14+$ monocytes. Arterioscler Thromb Vasc Biol 2005;25:1439–45.
62 Ruiz-Velasco N, Domínguez A, Vega MA. Statins upregulate CD36 expression in human monocytes, an effect strengthened when combined with PPAR-gamma ligands. Putative contribution of Rho GTPases in statin-induced CD36 expression. Biochem Pharmacol 2004;67:303–13.
63 Yilmaz A, Reiss C, Weng A *et al.* Differential effects of statins on relevant functions of human monocyte-derived dendritic cells. J Leukoc Biol 2006;79:529–38.
64 Seriolo B, Paolino S, Ferrone C, Cutolo M. Effects of etanercept or infliximab treatment on lipid profile and insulin resistance in patients with refractory rheumatoid arthritis. Clin Rheumatol 2007;26:1799–800.